CLAIMS

What is claimed is:

1. A host cell engineered to express at least one nucleic acid encoding a glycoprotein-modifying glycosyl transferase at a regulated level.

2. The host cell of Claim 1, wherein a nucleic acid molecule comprising at least one gene encoding a glycoprotein-modifying glycosyl transferase has been introduced in said host cell.

3. The host cell of Claim 1, wherein said host cell has been engineered such that an endogenous glycoprotein-modifying glycosyl transferase is activated.

4. The host cell of Claim 2 or 3, wherein said host cell is a CHO cell, a BHK cell, a NS0 cell, a SP2/0 cell, or a hybridoma cell.

5. The host cell of Claim 3, wherein said endogenous glycoprotein-modifying glycosyl transferase has been activated by insertion of a regulated promoter element into the host cell chromosome.

6. The host cell of Claim 2 or 3, wherein said glycoprotein-modifying glycosyl transferase is GnT III, GnT V, Man II, or Gal T.

7. The host cell of Claim 2 or 3, wherein said host cell is engineered to express at least two different glycoprotein-modifying glycosyl transferases selected from the group consisting of GnT III, GnT V, Man II, and Gal T.

8. The host cell of Claim 7, wherein at least one gene encoding a glycoprotein-modifying glycosyl transferase is operably linked to a constitutive promoter element.

9. The host cell of Claim 2, wherein at least one gene encoding a

glycoprotein-modifying glycosyl transferase is operably linked to a regulated promoter element.

10. The host cell of Claim 5 or 9, wherein the regulated promoter element is a tetracycline-regulated promoter system, an ecdysone-inducible promoter system, a lac-switch promoter system, a glucocorticoid-inducible promoter system, a temperature-inducible promoter system, or a metallothionein metal-inducible promoter system.

11. A host cell engineered to express at least one nucleic acid molecule encoding a glycoprotein-modifying glycosyl transferase, wherein said host cell is capable of producing a protein having enhanced Fc-mediated cellular cytotoxicity.

12. The host cell of Claim 11, wherein said protein is a whole antibody molecule, an antibody fragment, or a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin.

13. The host cell of Claim 12, wherein a nucleic acid molecule comprising at least one gene encoding a glycoprotein-modifying glycosyl transferase has been introduced in said host cell.

14. The host cell of Claim 12, wherein said host cell has been selected to carry a mutation triggering expression of an endogenous glycoprotein-modifying glycosyl transferase.

15. The host cell of Claim 14, wherein said host cell is the mutant lec10.

16. The host cell of Claim 12, wherein said host cell has been engineered such that an endogenous glycoprotein-modifying glycosyl transferase is activated.

17. The host cell of Claim 16, wherein said endogenous glycoprotein-modifying glycosyl transferase has been activated by insertion of a regulated promoter element into the host cell chromosome.

18. The host cell of Claim 16, wherein said endogenous glycoprotein-modifying glycosyl transferase has been activated by insertion of a constitutive promoter element, a transposon, or a retroviral element into the host cell chromosome.

19. The host cell of Claim 11 or 13, further comprising at least one transfected nucleic acid encoding an antibody molecule, an antibody fragment, or a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin.

20. The host cell of Claim 13, wherein at least one gene encoding a glycoprotein-modifying glycosyl transferase is operably linked to a constitutive promoter element.

21. The host cell of Claim 13, wherein at least one gene encoding a glycoprotein-modifying glycosyl transferase is operably linked to a regulated promoter element.

22. The host cell of Claim 21, wherein the regulated promoter element is a tetracycline-regulated promoter system, an ecdysone-inducible promoter system, a lac-switch promoter system, a glucocorticoid-inducible promoter system, a temperature-inducible promoter system, or a metallothionein metal-inducible promoter system.

23. The host cell of Claim 11, wherein said host cell is a hybridoma cell.

24. The host cell of Claim 11, wherein said engineered host cell is an engineered CHO cell, an engineered BHK cell, an engineered NS0 cell, or an engineered SP2/0 cell.

25. The host cell of Claim 11, wherein said host cell comprises at least one transfected nucleic acid encoding a chimeric anti-CD20 monoclonal antibody (C2B8).

26. The host cell of Claim 11, wherein said host cell comprises at least one transfected nucleic acid encoding a chimeric anti-human neuroblastoma monoclonal

antibody (chCE7).

27. The host cell of Claim 11, wherein said host cell comprises at least one transfected nucleic acid encoding a chimeric anti-human renal cell carcinoma monoclonal antibody (ch-G250), a humanized anti-HER2 monoclonal antibody, a chimeric anti-human colon, lung, and breast carcinoma monoclonal antibody (ING-1), a humanized anti-human 17-1A antigen monoclonal antibody (3622W94), a humanized anti-human colorectal tumor antibody (A33), an anti-human melanoma antibody (R24) directed against GD3 ganglioside, or a chimeric anti-human squamous-cell carcinoma monoclonal antibody (SF-25).

28. The host cell of Claim 11, wherein at least one nucleic acid molecule encodes ß(1,4)-N-acetylglucosaminyltransferase III (GnT III).

29. The host cell of Claim 28, further comprising at least one nucleic acid encoding a ß(1,4)-galactosyl transferase (GalT).

30. The host cell of Claim 28, further comprising at least one nucleic acid encoding a mannosidase II (Man II).

31. The host cell of Claim 28, further comprising at least one nucleic acid encoding a β(1,4)-galactosyl transferase (GalT) and at least one nucleic acid encoding a mannosidase II (Man II).

32. A method for producing a protein compound having enhanced Fc-mediated cellular cytotoxicity in a host cell, comprising:

(a) providing a host cell engineered to express a glycoprotein-modifying glycosyl transferase at a regulated level, chosen to improve glycosylation of a protein compound of interest, wherein said host cell expresses at least one nucleic acid encoding an antibody, an antibody fragment, or a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin;

(b) culturing said host cell under conditions which permit the production of said protein compound having enhanced Fc-mediated cellular

cytotoxicity; and

(c) isolating said protein compound having enhanced Fc-mediated cellular cytotoxicity.

33. The method of Claim 32, wherein in step (a), said host cell comprises at least one nucleic acid encoding a whole antibody.

34. The method of Claim 32, wherein in step (a), said host cell comprises at least one nucleic acid encoding an antibody fragment.

35. The method of Claim 32, wherein in step (a), said host cell comprises at least one nucleic acid encoding a fusion protein comprising a region resembling a glycosylated Fc region of an immunoglobulin.

36. The method of Claim 32, wherein said host cell comprises at least one transfected nucleic acid encoding a chimeric anti-CD20 monoclonal antibody (C2B8).

37. The method of Claim 32, wherein said host cell comprises at least one transfected nucleic acid encoding a chimeric anti-human neuroblastoma monoclonal antibody (chCE7).

38. The method of Claim 32, wherein said host cell comprises at least one transfected nucleic acid encoding a chimeric anti-human renal cell carcinoma monoclonal antibody (ch-G250), a humanized anti-HER2 monoclonal antibody, a chimeric anti-human colon, lung, and breast carcinoma monoclonal antibody (ING-1), a humanized anti-human 17-1A antigen monoclonal antibody (3622W94), a humanized anti-human colorectal tumor antibody (A33), an anti-human melanoma antibody (R24) directed against GD3 ganglioside, or a chimeric anti-human squamous-cell carcinoma monoclonal antibody (SF-25).

39. The method of Claim 32, wherein at least one gene encoding a glycoprotein-modifying glycosyl transferase has been introduced into said host cell.

40. The host cell of Claim 32, wherein said host cell has been selected to carry a mutation triggering expression of an endogenous glycoprotein-modifying glycosyl transferase.

41. The host cell of Claim 40, wherein said host cell is the mutant lec10.

42. The host cell of Claim 32, wherein said host cell has been engineered such that an endogenous glycoprotein-modifying glycosyl transferase is activated.

43. The method of Claim 32, wherein said glycosyl transferase is a ß(1,4)-N-acetylglucosaminyltransferase III (GnT III).

44. The method of Claim 43, wherein said GnT III is expressed using a constitutive promoter system.

45. The method of Claim 43, wherein said GnT III is expressed using a regulated promoter system.

46. The method of Claim 45, wherein said regulated promoter system is a tetracycline-regulated promoter system, an ecdysone-inducible promoter system, a lac-switch promoter system, a glucocorticoid-inducible promoter system, a temperature-inducible promoter system, or a metallothionein metal-inducible promoter system.

47 The method of Claim 32, wherein said glycosyl transferase is a β(1,4)-galactosyl transferase (GalT).

48. The method of Claim 47, wherein said GalT is expressed using a constitutive promoter system.

49. The method of Claim 47, wherein said GalT is expressed using a regulated promoter system.

50. The method of Claim 49, wherein said regulated promoter system is

a tetracycline-regulated promoter system, an ecdysone-inducible promoter system, a lac-switch promoter system, a glucocorticoid-inducible promoter system, a temperature-inducible promoter system, or a metallothionein metal-inducible promoter system.

51. The method of Claim 32, wherein said host cell is engineered to express a plurality of nucleic acids encoding a glycoprotein-modifying glycosyl transferase at a regulated level, chosen to improve glycosylation of a protein compound of interest, wherein at least one nucleic acid encodes GnT III and at least one nucleic acid encodes a β(1,4)-galactosyl transferase (GalT).

52. The host cell of Claim 51, wherein a nucleic acid molecule comprising at least one gene encoding a glycoprotein-modifying glycosyl transferase has been introduced into said host cell.

53. The host cell of Claim 51, wherein said host cell has been selected to carry a mutation triggering expression of at least one endogenous glycoprotein-modifying glycosyl transferase.

54. The host cell of Claim 51, 52, or 53, wherein said host cell has been engineered such that an endogenous glycoprotein-modifying glycosyl transferase is activated.

55. The method of Claim 32, wherein said host cell comprises a plurality of nucleic acids encoding a glycoprotein-modifying glycosyl transferase at a regulated level, chosen to improve glycosylation of a protein compound of interest, wherein at least one nucleic acid encodes GnT III and at least one nucleic acid encodes a mannosidase II (Man II).

56. The host cell of Claim 55, wherein a nucleic acid molecule comprising at least one gene encoding a glycoprotein-modifying glycosyl transferase has been introduced into said host cell.

57. The host cell of Claim 55, wherein said host cell has been selected to

carry a mutation triggering expression of at least one endogenous glycoprotein-modifying glycosyl transferase.

58. The host cell of Claim 55, 56, or 57, wherein said host cell has been engineered such that an endogenous glycoprotein-modifying glycosyl transferase is activated.

59. The method of Claim 32, wherein said host cell comprises a plurality of nucleic acids encoding a glycoprotein-modifying glycosyl transferase at a regulated level, chosen to improve glycosylation of a protein of interest, wherein at least one nucleic acid encodes GnT III, at least one nucleic acid encodes β(1,4)-galactosyl transferase (GalT), and at least one nucleic acid encodes mannosidase II (Man II).

60. The host cell of Claim 59, wherein a nucleic acid molecule comprising at least one gene encoding a glycoprotein-modifying glycosyl transferase has been introduced into said host cell.

61. The host cell of Claim 59, wherein said host cell has been selected to carry a mutation triggering expression of at least one endogenous glycoprotein-modifying glycosyl transferase.

62. The host cell of Claim 59, 60, or 61, wherein said host cell has been engineered such that an endogenous glycoprotein-modifying glycosyl transferase is activated.

63. The method of Claim 32, wherein the expression level of at least one glycoprotein-modifying glycosyl transferase has been selected to produce an antibody molecule, an antibody fragment, or a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin having enhanced Fc-mediated cellular cytotoxicity at a higher level than the Fc-mediated cellular cytotoxicity obtained from a different expression level of the same glycosyl transferase gene.

64. The method of Claim 63, wherein said expression levels are

determined by Western blot analysis using a glycosyl transferase-specific antibody.

65. The method of Claim 63, wherein said expression levels are determined by Northern blot analysis using a glycosyl transferase-specific probe.

66. The method of Claim 63, wherein said expression levels are determined by measuring the enzymatic activity of glycosyl transferase.

67. The method of Claim 63, wherein said expression levels are determined using a lectin which binds to biosynthetic products of glycoprotein-modifying glycosyl transferase.

68. The method of Claim 67, wherein the lectin is E_4-PHA lectin.

69. The method of Claim 63, wherein said nucleic acid encoding said glycoprotein-modifying glycosyl transferase is operatively linked to a reporter gene, and wherein said expression levels of said glycosyl transferase are determined by measuring a signal correlated with the expression level of said reporter gene.

70. The method of Claim 69, wherein said reporter gene is transcribed together with at least one nucleic acid encoding said glycoprotein-modifying glycosyl transferase as a single RNA molecule and their respective coding sequences are linked either by an internal ribosome entry site (IRES) or by a cap-independent translation enhancer (CITE).

71. The method of Claim 69, wherein said reporter gene is translated together with at least one nucleic acid encoding said glycoprotein-modifying glycosyl transferase such that a single polypeptide chain is formed.

72. The method of Claim 63, wherein said nucleic acid encoding said glycoprotein-modifying glycosyl transferase is operatively linked to a reporter gene under the control of a single promoter, wherein said nucleic acid encoding said glycoprotein-modifying glycosyl transferase and said reporter gene are transcribed into

an RNA molecule which is alternatively spliced into two separate messenger RNA (mRNA) molecules, wherein one of the resulting mRNAs is translated into said reporter protein, and the other is translated into said glycoprotein-modifying glycosyl transferase.

73. The method of Claim 32, wherein said host cell further comprises a nucleic acid encoding a glycosidase.

74. An antibody having enhanced antibody dependent cellular cytotoxicity (ADCC) produced by the host cells of Claim 11.

75. A chimeric anti-CD20 monoclonal antibody (C2B8) having enhanced antibody dependent cellular cytotoxicity (ADCC) produced by the host cells of Claim 25.

76. A chimeric anti-human neuroblastoma monoclonal antibody (chCE7) having enhanced antibody dependent cellular cytotoxicity (ADCC) produced using the host cells of Claim 26.

77. A chimeric anti-human renal cell carcinoma monoclonal antibody (ch-G250) having enhanced antibody dependent cellular cytotoxicity (ADCC) produced using the host cells of Claim 27.

78. A humanized anti-HER2 monoclonal antibody having enhanced antibody dependent cellular cytotoxicity (ADCC) produced using the host cells of Claim 27.

79. A chimeric anti-human colon, lung, and breast carcinoma monoclonal antibody (ING-1) having enhanced antibody dependent cellular cytotoxicity (ADCC) produced using the host cells of Claim 27.

80. A humanized anti-human 17-1A antigen monoclonal antibody (3622W94) having enhanced antibody dependent cellular cytotoxicity (ADCC)

produced using the host cells of Claim 27.

81. A chimeric anti-human squamous-cell carcinoma monoclonal antibody (SF-25) having enhanced antibody dependent cellular cytotoxicity (ADCC) produced using the host cells of Claim 27.

82. A humanized anti-human colorectal tumor antibody (A33), having enhanced antibody dependent cellular cytotoxicity (ADCC) produced using the host cells of Claim 27.

83. An anti-human melanoma antibody (R24) directed against GD3 ganglioside, having enhanced antibody dependent cellular cytotoxicity (ADCC) produced using the host cells of Claim 27.

84. An antibody fragment that includes a region equivalent to the Fc region of an immunoglobulin, having enhanced Fc-mediated cellular cytotoxicity produced using the host cells of Claim 11.

85. A fusion protein that includes a region equivalent to the Fc region of an immunoglobulin, having enhanced Fc-mediated cellular cytotoxicity produced using the host cells of Claim 11.